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IN VITRO SELECTION BASAL MEDIUM USED FOR SEEDS GERMINATION, CALLOGENESIS INDUCTION OF LOCAL VARIETIES OF VICIA FABA L

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Abstract:

One of the most significant winter crops consumed by humans in the Middle East is the faba bean (Vicia faba L.). As many other vetches and field crops.
The ability of this plant to withstand such a biotic stress may be improved with the help of tissue culture.
This investigation was carried out to create and selection basal medium used for seeds germination, callogenesis induction in three Faba bean varieties, Baraqat, Iraqi and Halabi, extensively grown for personal consumption in Iran, Iraq, and Syria respectively.
First seeds were sterilized and planted on a growth-regulatory-free MS basal medium. Germination rates ranked between 24.6 to 63% because of the lethal effect of phenolic compounds.
Five types of explants excised on the in vitro obtained seedlings were used for callus initiation: stems, shoot tips, leaves, epicotyls, and parts of the roots. They were put on MS basal medium that had been increased by BAP at a dosage of 0.5, 1.0, or 1.5 mg/l.. Depending on the genotypes, explants, and medium, callogenesis rates ranged from 33.33 to 100%. In terms of callogenesis rate and callus weight, shoot tip segments grown on media with BAP at 1 and 1.5 mg/l generated the highest outcomes of all three kinds. Even though it wasn't the goal of this study, the creation of somatic embryos was conceivably achievable (33%) throughout this study and happened just 2 months after calluses started, suggesting the embryogenic ability of such calluses for the 3 kinds examined.
Keywords: Faba Bean, Landraces, Callogenesis.

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Introduction:

One of the first domesticated crops was the faba bean (Vicia faba L.). (Duke, 2012). Remaining seed from the 10th Millennium before Present (BP) has been found in northern Syria, supporting the theory that the crop originated in the Near East. Later, it spread to China, North Africa, and Europe. For millennia, it has been grown in South America and Australia, respectively. (Kosev *et al,* 2021).

Faba one of the most significant legume crops in the globe is the bean. It is a significant crop grown to use for personal consumption and animals nutrition since it is a great source of protein. (Maalouf, 2019). Faba beans are consumed as fresh buds and fresh and dry seeds. This staple crop is expecting increasing importance in the future for both human food and animal feed. Farmers will find this plant more alluring due to its high production, smaller seeds, fewer antinutritional elements, and good adaptability to contemporary agriculture, in addition to the longevity of storage life. Additionally A major contributor to the biological fixing of atmospheric nitrogen is the faba bean and is usually exploited as green manure (Duke, 2012).

With a global yield of over 4.5 million tons in 2004, this species alone occupied nearly 3.2 million acres in 1991 (FAQSTAT, 2011). Despite being grown in numerous nations, China produces 49% of the world's faba beans, followed by Egypt (10.1%), Ethiopia (8.6%), and Australia (5.9%). In the Near East, Faba bean is widely cultivated in Egypt, Iran, Iraq, Saudi Arabia, Sudan, Syria, Turkey, for both food and feed uses (FAOSTAT, 2011).

MATERIAL AND METHODS

Plant material

Three landraces of Faba bean commonly cultivated in the Arab Golf region for human consumption have been used in this study: *Iraqi,* given by Abaa Center for Agricultural Research of Iraq; *Halabi* from Aleppo, Syria; and *Baraqat*, an Iranian variety delivered by the Agriculture Biotechnology Research Institute of Iran.

Seeds germination

Afterwards, seeds were sterilized in 30% hydrogen peroxide H2O2 after being softened in water for 24 hours for 10 minutes, then 20% commercial bleach for 20 minutes, followed by three to four rinses with sterile distilled water. Sterilized seeds were sown on MS basal medium (Neumann *et al,* 2009) deprived of growth regulators. Regular control was undergone to eliminate oxidized explants and the ones contaminated with fungi.

Callogenesis induction

Five types of explants (1 cm) have been excised from the *in vitro* germinated seeds (21 days) and used for callus initiation: i) epicotyl segments ; ii) stem segments ; iii) shoot tips; iv) leaves; v) root segments. These explants were scarified and cultivated onto MS basal medium supplemented with BAP 0.5, 1.0, or 1.5 mg/l. Parameters such as callogenesis rate, callus weight and callus rate producing somatic embryos were examined.

Cultures growth conditions

A 16/8 h light/dark photoperiod regime with white fluorescent light at 716 Lux was used to incubate the cultures at a temperature of 25 °C ±1.

RESULTS AND DISCUSSION

1. Seeds Germination

Few days after cultures initiation, plated seeds of the three faba landraces *Iraqi*, *Halabi* and *Baraqat*, faced the occurrence of fungal contamination and phenolic compounds that quickly invaded the medium which led to the cells death and seeds loss. Globally the average rate of surviving seeds didn't exceed 42 % (Table 1). The best rate of germinated seeds was recorded for *Iraqi* (63%) while *Halabi* and *Baraqat* had low germination rates of only 38.8 and 24.6 % respectively. Although modest, seeds germination rates were enhanced by incorporating citric and ascorbic acids into the medium to prevent the production or oxidation of the phenolic compounds as previously reported in faba bean (Bahgat *et al,* 2008).

2. Callogenesis

Epicotyls, stems, shoot tips, leaves, and root segments were among the five types of explants removed from the germinated seeds and utilized for callus initiation on MS medium supplemented with BAP 0.5, 1.0, or 1.5 mg/l. Eight to nine days later, white callus started to occur on the wounded explants. Callus color changed progressively to greenish as to its development (Fig. 1). It is worthy to note that calluses were subcultured every two weeks onto fresh new media in order to reduce the negative impact of phenolic compounds exudates but also to allow an appropriate callus.

All type of the explants tested for the three landraces were able to develop calluses with however a rate varying between 33.33 to 100% according to the landraces, explants and media. Furthermore, calluses proliferation through time varied significantly according to the same factors (Table 3).

Globally callus average weight recoded after two months ranked between a minimum of 110 mg for *Halabi* epicotyl callus on BAP 0.5 mg/l to 718 mg for shoot tip callus of the same genotype obtained on BAP 1 mg/l. As to the medium, BAP 0.5 mg/l was significantly less effective on the callogenesis rate and proliferation while BAP 1 or 1.5 mg/l led significantly to better results. A significant interaction was recorded between the different factors (genotype x explant x medium). Actually for the three landraces, the best results were recorded for shoot tip segments cultivated on media containing BAP at 1 and 1.5 mg/l, in terms of callus weight and callogenesis rate. It is worthy to note that production of somatic embryos, within two months of the start of the calluses, an unforeseen outcome of this study was hoped to be achievable during this experiment, with a low rate of 33%.

These results are in line with previous ones reported on the callogenesis establishment by using Cotyledonary nodes of four Polish cultivars' immature seeds were gathered. (Skrzypek *et al.,* 2012) and some Egyptian varieties (Bahgat *et al.*, 2008). Medium optimization and selection of growth regulators are a key issue to maintain viable callus in culture and to induce the production of somatic embryos (Khilwani *et al.,* 2016).

This is the first report on callogenesis of three faba bean varieties cultivated in the Middle East Iraqi, Halabi, Baraqat and considered as landraces in Iraq, Syria and Iran respectively.

CONCLUSIONS

For that end, technical steps were adjusted starting by seeds germination to the callus establishment.

Oxidation was the major constraint facing the whole process but it was partially resolved by the incorporation of antioxidants (citric and ascorbic acids) into the culture media. Although seeds germination rates were low (24.6 to 63.8%), they were enough to initiate aseptically cultures for the subsequent steps.

Callogenesis was achieved for the three genotypes with rates varying between 33.33 to 100% according to the genotypes and explants types. The explants type and BAP concentration were a key issue to maintain the calluses and to induce their proliferation as

suggested in previous works. The best results in terms of callogenesis rate and callus weight were recorded for shoot tip segments with BAP adjusted at 1 to 1.5 mg/l.

Though unanticipated in this work, somatic embryo development appeared to be feasible throughout this experiment, happening two months after calluses were initiated, with a low rate of 33%, thereby indicating the embryogenic potential of these calluses. It will be further interesting of developing embryogenesis as previously experimented in other faba bean genotypes (Djilianov *et al.*, 2013).

These preliminary results are particularly promising and incite to develop further steps beyond this work towards:

Experimenting *in situ* conditions the behavior of regenerated plantlets under salt stress.

Tables

Table 1. Seeds germination rate of three faba landraces on MS medium after two weeks of culture initiation (100 explants per landrace).

Landrace	Seeds lost by fungal contamination %	Seeds lost by phenolic compounds %	Germinated seeds %
Iraqi	**19.4 c**	**16.6 d**	**63.8 a**
Halabi	**16.6 c**	**44.4 b**	**38.8 b**
Baraqat	**30.5 b**	**13.8 d**	**55.7 a**
Average	**22.1**	**24.9**	**52.7**

- At a probability threshold of 0.05, values denoted by different letters differ significantly from each other. (Duncan test).

Table 2. Basal medium used for seeds germination, callogenesis induction.

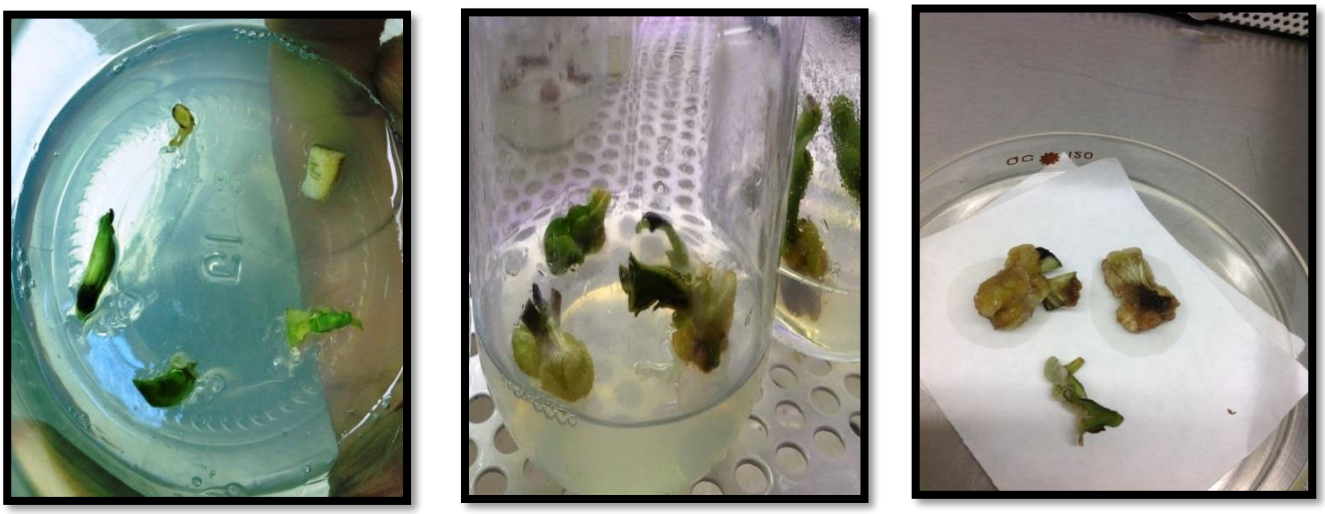
Macro-elements	Composition in mg/l of Neumann *et al,* 2009
NH_4NO_3	16 50
KNO_3	1900
$MgSO_4.7H_2O$	370
KH_2PO_4	170
$CaCl_2.2H_2O$	440
Micro-elements	Composition in mg/l Neumann *et al,* 2009
KI	0.83
H_3BO_4	6.2
$MnSO_4.H_2O$	15.1
$ZnSO_4.7H_2O$	8.6
$NaMoO_4.2H_2O$	0.25
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
Fer	mg/l
$FeSO_4.7H_2O$	27.8
$NaEDTA.H_2O$	37.3
Vitamines	mg/l
Myo Inositol	100
Acide nicotinique	0.5
Pyridoxine	0.5
Thiamine	1
Glycine	0.2
Glutamine	200
Acide Folic	-
Biotine	-
Others	
Saccharose	30 g/l
Distilled water	up to 1000 ml
pH	5.7
Agar	6.5 g/l
Autoclaving	118°C, 20 minutes
Ascorbic acid	50 mg/l
Citric acid	50 mg/l
pH	6

Figures

Figure 1. Embryogenic calluses obtained six weeks after initiation on BAP 1.5 mg/l.

figure 2.

Callogenesis establishment of faba bean var. Halabi on MS supplemented with BAP 1 mg/l. (A) Initiation from different explants; (B,C) Callus established from stem segments.



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